AN IMMUNE-ENRICHED OLIGO-MICROARRAY ANALYSIS OF GENE EXPRESSION IN MANILA CLAM (*Venerupis philippinarum*) HAEMOCYTES AFTER A *Perkinsus olseni* CHALLENGE

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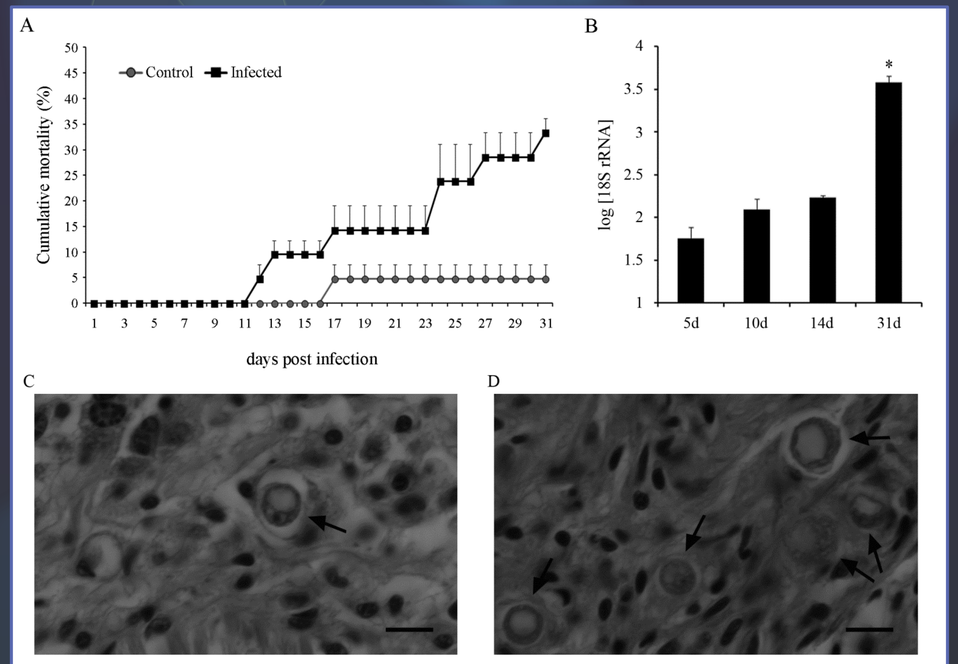
INTRODUCTION AND OBJECTIVES :

The Manila clam (*Venerupis philippinarum*) is one of the most extensively cultured bivalves in the world, especially in Asia and Europe. Parasites of the genus *Perkinsus* cause high mortality and economic losses in bivalves commonly produced in global aquaculture. Although the immune responses of oysters and clams naturally infected with *P. marinus* or *P. olseni* have been extensively studied, there is not much information on host response at the early stages of infection. Oligo-microarrays are a sensitive and reproducible technology for studying complex biological functions. This technology has been used to analyse gene expression in *C. virginica* infected with *P. marinus* and in *V. decussatus* naturally infected with *P. olseni*.

In this study, we used an immune-enriched DNA microarray to analyse how *P. olseni* influences the gene expression profiles of haemocytes from Manila clams intramuscularly injected with this pathogen. Our results provide novel observations of the innate immune response at different infection stages.



EXPERIMENTAL INFECTION AND MORTALITIES

Animals were im infected with *Perkinsus* trophozoites ($5x10^3$ trophozoites) and maintained in 50-l tanks at 22 °C.

(A) A **12-day period with no mortalities** was followed by a **constant increase** to the end of the experiment.

(B) The **parasite load** was quantified by qPCR. An increase in the parasite 18S gene mRNA was registered.

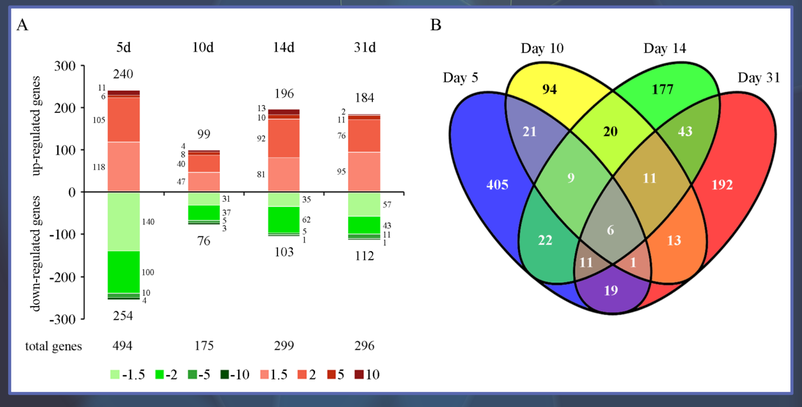
(C-D) Presence of a few trophozoites in the **mantle (14)** and **connective tissue (31)**. It was **not detected at 5 and 10 d.**

MICROARRAY HYBRIDISATION, ROBUSTNESS AND VALIDATION

Haemolymph from 20 animals were sampled at 5, 10, 14, and 31 days post-infection and RNA extracted.

A **8x15K Agilent oligo-microarray** was used. Fluorescence values were deposited in the **GEO database (GSE GSE59399).**

Results were **validated by qPCR** and confirmed the expression patterns of four selected genes



GENERAL RESPONSE OF HAEMOCYTES TO *P. olseni* INFECTION

(A) Distribution of the **number of genes regulated** throughout infection.

(B) Venn diagram of differentially expressed genes in haemocytes at the four sampling times after infection

GENE EXPRESSION PROFILE AFTER *P. olseni* INFECTION (HIGHLIGHTS)

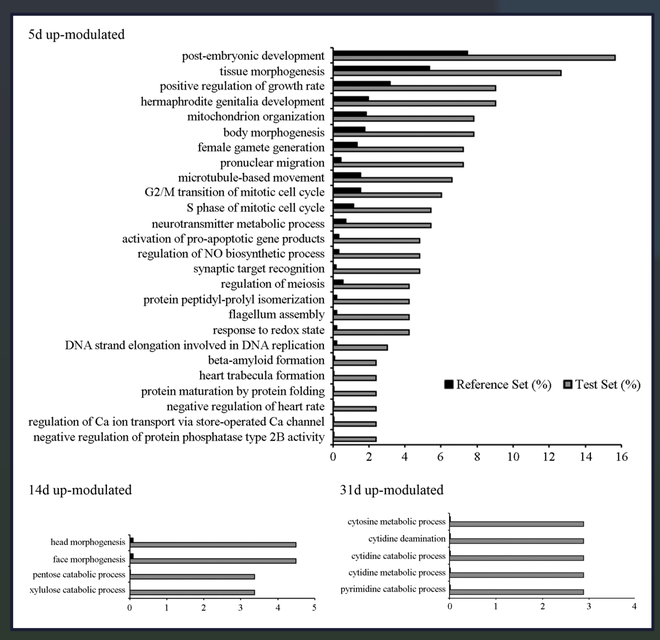
Three phases of infection in clam haemocytes.

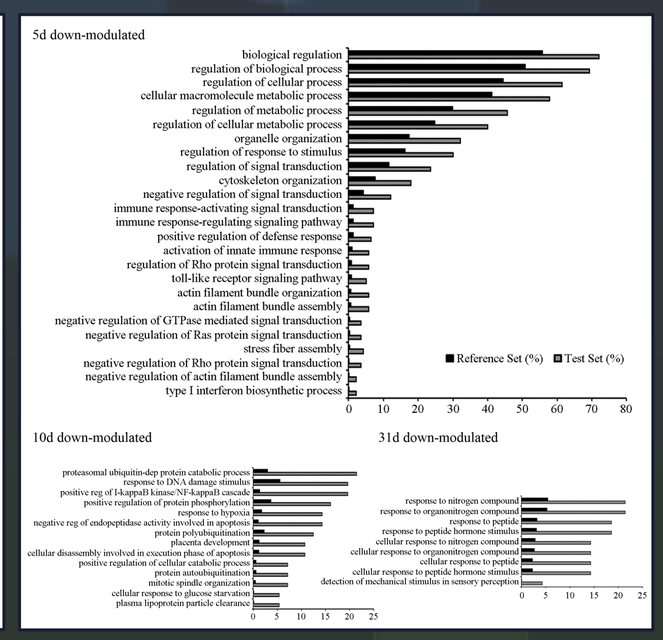
The **early phase** was characterised by **no mortality** and by the increased expression of genes associated with **pathogen recognition, production of N radicals and antimicrobial activity**. Cellular processes such as inhibition of serine proteases and proliferation were also involved in this early response.

At **intermediate stage**, when the animals began to **die**, many genes related to **cell movement** were over-expressed.

At **late stage** (30 days) **metabolic pathway** genes were the most affected at thirty days after infection.

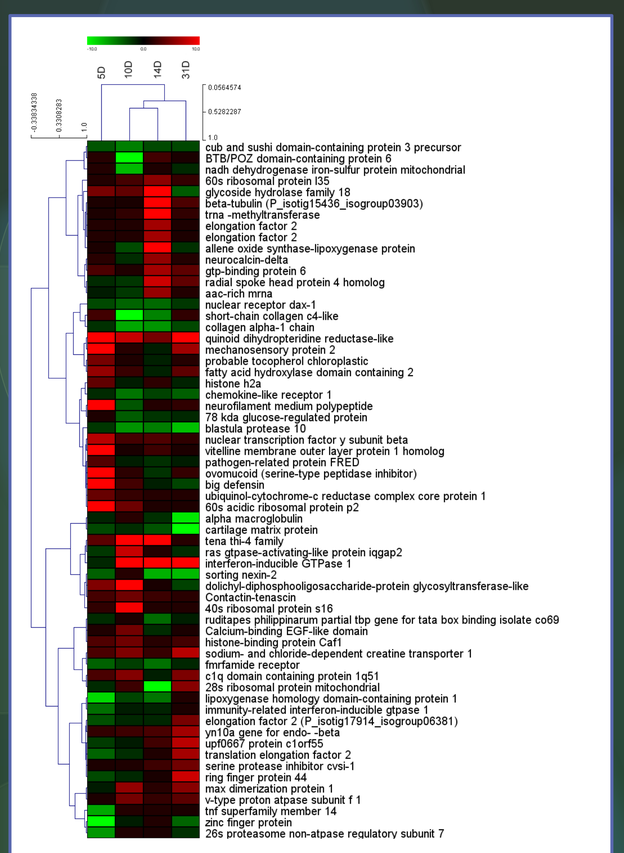
Apoptosis appears to be important during pathogenesis.



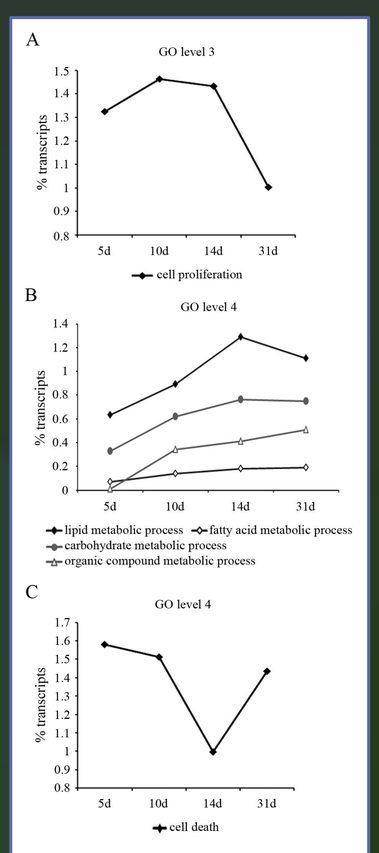


ENRICHMENT ANALYSIS

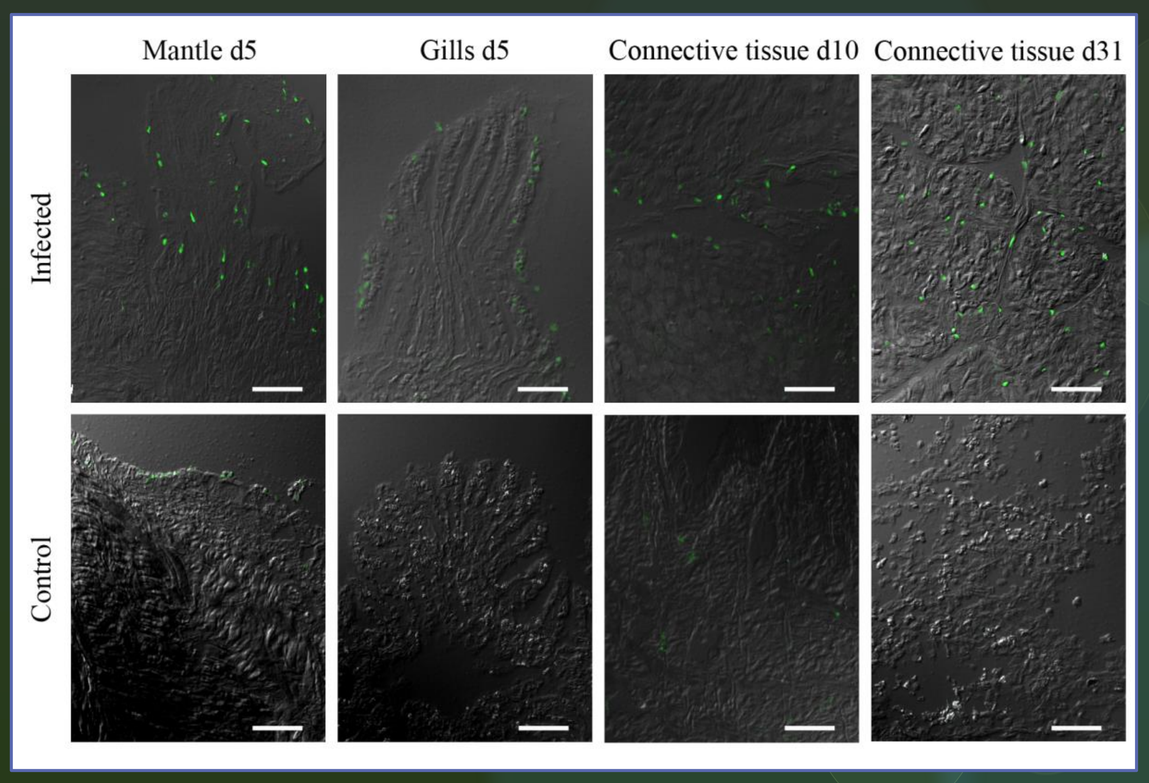
Distribution of GO terms up and down-regulated between the test set (significantly expressed genes for each sampling point) and the reference set (all of the sequences present in the microarray).



Heat map showing the **evolution of differentially expressed genes** with fold changes values higher that 5 at the different sampling points



Percentage of transcripts included in the GO categories associated with cell proliferation (A), metabolic processes (B) and apoptotic cell death (C) at the different sampling points.



APOPTOSIS DURING *P. olseni* INFECTION

TUNEL assay was conducted in histological sections.

High number of apoptotic cells was detected in the **mantle and gills at day 5** and in **connective tissues** between muscular fibres surrounding the digestive gland at later sampling points (**day 14 and 31**).